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Molecular pathology of hereditary cerebral hemorrhage with amyloidosis-Dutch type

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Citation

Grand Moursel, L. (2019, November 21). *Molecular pathology of hereditary cerebral hemorrhage with amyloidosis-Dutch type*. Retrieved from <https://hdl.handle.net/1887/80759>

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Issue Date: 2019-11-21

Chapter 7

Discussion

Aim of the study & central findings

The general aim of this thesis was to decipher the molecular pathogenesis of HCHWA-D. Since no proven therapeutic treatment exists to prevent or even delay the disease onset, the understanding of underlying pathomechanisms in HCHWA-D is important. It may help discovering new therapeutic targets and biomarkers that can be used to assess the efficacy of candidate drugs in treatment trials.

The main finding of the work presented in this thesis is that TGF β deregulation plays a central role in HCHWA-D pathology. This deregulation is demonstrated using HCHWA-D brain material. At the gene expression level, on individual genes, TGF β 1, its receptors and TGF β -induced genes are upregulated (chapter 3). At the pathway level, in the transcriptomic study, extracellular matrix (ECM)-pathways related to TGF β are increased (chapter 6). Lastly at the protein level using histology, we showed the actual activation of the TGF β signaling cascade. First by showing the accumulation of its activated transcription factor (pSMAD2/3; chapter 3) in some angiopathic vessels, then by identifying the presence of the pro-fibrotic collagen 1 (col1) protein, a major TGF β -induced genes, in amyloid-laden vessels (chapter 5). By selecting patients at different disease stages, we discovered that pSMAD2/3 and col1 accumulation in angiopathic vessel walls are correlated with CAA load (chapter 3 & 5).

We show that TGF β deregulation correlates with disease severity. However, disease severity in HCHWA-D is also characterized by the higher occurrence of secondary remodelling of angiopathic vessels (1). In an initial study, we identified vascular calcifications, a specific secondary vascular remodelling, to be the cause of a recently described neuroimaging feature of advanced disease stage in HCHWA-D (2). In the subsequent study, we assessed the presence of osteopontin (OPN) in these calcifications, as described in other non-A β related vasculopathies in link with TGF β increase (3). We further discovered a topographical association of pSMAD2/3 and OPN in these calcifications and we also linked their gradual accumulation with the calcification process (chapter 5).

Study of pathomechanisms in human brain tissue material

Advantages & Difficulties

The use of human brain material is undeniably a unique chance to study pathomechanisms in relevant tissue and has a high scientific value. Using patient tissue gives access to the whole complexity of the disease in comparison to pathological processes *in vitro* which lack the cellular and physiological context or *in vivo* studies in animal model which cannot fully recapitulate human brain diseases.

Despite these advantages, patient brain material is underused in research mostly because its utilization remains challenging (4). The principal drawback is the heterogeneity of samples due to individual differences (age, gender, health, body mass, life style), prior medication exposure and additional co-morbidities, which are confounding factors. Secondly, for gene expression analysis, the RNA quality is variable, mostly due to the circumstances of death (agonal stress, (5)) but other factors such as postmortem interval, dissection protocols and storage conditions can influence the RNA integrity as well. Because the RNA quality is generally lower than for RNA isolated from cell cultures or animal models, it requires testing and optimisation of standard procedures, as discussed in chapter 6. Thirdly, the material is scarce, which might be restrictive in experimental design. Lastly, postmortem human tissue reflects the end-stage of the disease and therefore cannot replace cellular and animal research models where early events in illness can be studied.

Frontal versus occipital pathology in HCHWA-D

Previous CAA neuropathological studies suggested that CAA pathology was more prominent in the occipital lobes than in the frontal lobes (chapter 1). We studied these two areas as they were presumed to represent different disease stages. Some histopathological findings (other than β -amyloid vascular accumulation) were indeed more prominent occipitally. We discovered both perivascular ring of pSMAD2/3 granules (chapter 3) and cortical calcifications of angiopathic vessels (which are a very advanced disease stage) predominantly occipitally (chapter 4), although a direct link between these two findings remain to be demonstrated. However, at the transcriptomic level, we did not find evidence that frontal and occipital cortexes of HCHWA-D were differently affected (chapter 6). Also the amount of angiopathic vessels showed no significant difference between the frontal and the occipital cortex (chapter 3). Nevertheless, one finding of this thesis is that the study of patients with different disease stages is more informative of pathomechanisms than the study of different brain areas.

The correlation of the different CAA load with pSMAD2/3 accumulation independently of the brain area, revealed TGF β -related pathomechanisms (chapter 3).

- Pathological disease severity in HCHWA-D is comprised of CAA load plus additional secondary vascular remodeling
- Gene expression in frontal and occipital cortex is similar but secondary vessel pathology showed more perivascular changes and vascular calcifications occipitally

Consequences of TGF β activation

TGF β s are pleiotropic cytokines involved in a variety of biological effects, modulating ECM metabolism, cell cycle as well as immune response (6). Transgenic mice overexpressing TGF β 1 in brain cells have been extensively studied to understand the influence of this cytokine on the vasculature, on neuroinflammation and on A β deposition (7,8). Results of this thesis, in light of knowledge from previous neuropathological and transgenic mice studies, further implicate TGF β in HCHWA-D phenotypic variability.

Effects of TGF β on the cerebrovasculature

Cerebrovascular fibrosis

The pathogenesis of cerebrovascular fibrosis has been studied in mouse model overexpressing TGF β 1 in astrocytes (GFAP-TGF β 1, (9)). It starts by a perivascular astrogliosis inducing an early thickening of vascular basement membranes (3-4 months) by ECM proteins, followed by the deposition of amyloid fibrils by 6 months of age. When these mice are crossed with mice overexpressing human APP (hAPP) in neurons (GFAP-TGF β 1/PDGF-hAPP), amyloid vascular deposits are detected even earlier (2-3 months). Remarkably, another mouse model with a neuron-specific TGF β 1 inducible overexpression, develop a similar angiopathic phenotype via induction of perivascular astrogliosis, confirming that astrocytes are major player in the cerebrovascular fibrosis (10).

Prior studies in HCHWA-D also described perivascular astrogliosis associated with arteriolar CAA (11) and perivascular ECM remodelling (12,13). Although the chronology of events is unknown, based on animal model studies, a TGF β 1-mediated fibrosis, preceding and triggering the CAA pathology might be an early event in HCHWA-D patients as well.

Accordingly, our transcriptomic study suggests an early TGF β 1-mediated fibrosis. By comparing the human transcriptome with the transcriptome of young APP-E693Q mice at the pathway level, we could identify an overlap

in the upregulation of ECM-related pathways, which is in mice prior to the onset of CAA pathology (chapter 6). Moreover, we confirmed in patient material the increase of TGF β -induced pro-fibrotic target genes such as plasminogen activator inhibitor-1 (PAI-1), fibronectin (FN1) and collagen (Type I Col1A1 and Type III Col3A1; chapter 3). We further proposed that the accumulation of Col1 protein in the vessel walls might be prior to amyloid deposition (chapter 5), suggesting a TGF β 1-mediated fibrosis triggering the CAA pathology.

Secondary vascular remodelling

Prolonged TGF β 1 overexpression in astrocytic-TGF β 1 mice results in degenerative changes in microvascular cells (at 9-18 month) and cerebral microhemorrhages at older age (18-24 month). Our results show that TGF β deregulation correlates with disease severity. As most severe cases are in general the oldest patients, it suggests that also in patients TGF β activation is prolonged, and is likely modulating the CAA pathology. The most severe cases also present with secondary vascular remodeling such as vascular calcifications studied in this thesis. Interestingly, in the neuronal-TGF β 1 mice, a microarray analysis of cortical gene expression showed upregulation of OPN as well as various genes also involved in tissue mineralization and vascular calcification, strengthening the influence of TGF β 1 on this type of vasculopathy.

Effect of TGF β on microglia / macrophages phenotype

TGF β is also a major player in the inflammatory phenotype of brain microglia and macrophages. The inflammatory response can present either two or three states: a pro-inflammatory state (M1) and a repair/resolution state (M2), which is sometimes subdivided into an alternative inflammation state (phagocytic, fibrotic) or an acquired deactivation state (immunosuppressive) (14). The M2 state is associated with, and induced by, a TGF β 1 increase (15). Although not extensively studied in this thesis, the immune response in HCHWA-D is thought to be influenced by TGF β 1 upregulation and to modulate the phenotype.

TGF β -promoted parenchymal clearance of A β by microglia activation is a probable underlying mechanism in HCHWA-D. Previous neuropathological studies in HCHWA-D described a tendency for a disappearance of parenchymal amyloid plaques with age (16) and identified microglial phagocytosis of non-fibrillar A β (11). In APP-overexpressing mice, in parallel to the increase of CAA pathology, additional TGF β 1 overexpression resulted in a microglial activation promoting the clearance of A β and reducing the amount of A β parenchymal plaques. It is suggested that TGF β 1 induces microglial phagocytosis (8) which is characteristic of M2 inflammatory

state. In HCHWA-D patients, typical M2 genes *Sphk1*, *TGFβ1* and *HO-1* (acquired deactivation) together with chitinase-Like Proteins genes *CHI3L2* and *CHI3L1* (alternative inflammatory, (14)) were significantly increased in our RNAseq study (chapter 6, supplementary tables).

Lastly, inflammatory CAA can also be induced by *TGFβ* pathway activation. Inflammatory CAA is characterized by the infiltration of macrophages and other blood-derived immune cells around and/or within the vessel walls of CAA-affected blood vessels. Perivascular macrophages have a function in the regulation of CAA (17) and *TGFβ1* is a central player in the modulation of the phenotype and brain infiltration of macrophages. Indeed, in APP and *TGFβ1*-overexpressing mice, induction of inflammatory CAA via activation of macrophages reduces the CAA pathology and is thus considered as beneficial (18,19). At histopathological examination, inflammatory CAA is common in HCHWA-D and is more frequently detected in patients with the most severe phenotype (20). Whether inflammatory CAA is beneficial or detrimental to the patients is a matter of debate. Although it is associated with diverse vasculopathies and increased risk of dementia (16), patients with inflammatory CAA survive on average longer and have smaller non-fatal hemorrhages (M. Maat Schieman, unpublished work). In conclusion, the exact contribution of these immune cells to the CAA pathology is unclear, limited to animal models studies and requires further investigations.

- The *TGFβ* pathway is activated in HCHWA-D, and contributes to the phenotypic variability
- *TGFβ* chronic overexpression aggravates the vascular fibrosis, is associated with the CAA pathology severity and might promote vascular calcification

Possible causes of *TGFβ* activation in HCHWA-D

TGFβ in the central nervous system (CNS) is induced upon injuries as part of the healing process. Independently of stroke-related brain injuries, we discuss below potential early *TGFβ* induction in HCHWA-D triggered by cellular stress or interference with *TGFβ* related processes.

Early response to cellular toxicity

Oligomeric *Aβ* species (o*Aβ*s) have been extensively studied and are the most toxic of the *Aβ* species accumulating in AD and in APP-overexpressing animal models. o*Aβ* are potent neurotoxins inducing a wide range of cellular

dysfunctions (oxidative and ER stress, calcium dyshomeostasis, cell cycle re-entry, mitochondrial dysfunction) as well as neurodegenerative reactions (21,22).

A higher stability of o*Aβ*s due to the E22Q mutation (chapter 1) have been predicted *in silico* (23), demonstrated *in vitro* (24–26) and further found *in vivo*, in APP-E693Q mice (27). In APP-E693Δ mice, a closely related animal model with a lack of glutamate at position 22 of *Aβ*, intraneuronal o*Aβ*s lead to mitochondrial dysfunction (28). Even though the actual existence of o*Aβ*s has never been proven in patients, we also found a mitochondrial dysfunction in our HCHWA-D transcriptomic study (chapter 6) indicating cellular stress, possibly triggered by toxic oligomeric species.

While less investigated, o*Aβ*s can elicit a stress response in other cell types than neurons. Particularly, endothelial cells (ECs) *in vitro* are highly susceptible to Dutch o*Aβ* peptide (29) and endothelial injuries lead to a CAA phenotype associated with *TGFβ1* upregulation (30). Nevertheless Dutch o*Aβ* addition to ECs in monoculture leads to a decrease in *TGFβ1* (31,32), and it is likely that the *TGFβ* response observed *in vivo* mostly comes from other cell type such as glial cells (33). Especially reactive astrocytes are susceptible to o*Aβ*s toxic species and can induce a neuroprotective *TGFβ* response. A recent study revealed that o*Aβ* can activate astrocytes; astrocyte-derived *TGFβ1* prevents synapse loss induced by o*Aβ* in AD mice (34). Such a protective astrocytic reaction to limit the level of neurotoxic soluble o*Aβ* was also suggested to occur in HCHWA-D (11).

Direct interferences with *TGFβ* activity

Finally, the Dutch mutation could directly interfere with *TGFβ*-related processes.

TGFβ1 co-aggregates with amyloid parenchymal plaques in AD and Down Syndrome, suggesting a neuroprotective *Aβ* sequestration (35). It was demonstrated that *Aβ* can bind to *TGFβ* (36) and to *TGFβ* receptors (*TGFβR*) (37). Although the effect of the Dutch *Aβ* peptide on the interaction with *TGFβ* is unknown, the FAQD Dutch *Aβ* motif was predicted to influence the *Aβ* binding to *TGFβ* receptors, meaning a putative direct interference with the signalling pathway.

Furthermore, Amyloid Precursor Protein (APP) and *TGFβ1* play a role in the coagulation cascade. APP is able to form complex with coagulation factors *in vivo*, and in blood of HCHWA-D patients the complex APP-factors XIa was slightly increased (38). Although the exact impact of the Dutch mutation on the coagulation cascade remains to be explored, APP is accumulating in HCHWA-D vascular amyloid (39) and might be blood-derived from platelets, which are rich in APP proteins. In transgenic CAA mouse models, platelet recruitment to vascular amyloid is prolonged (in

APP23 and APP Dutch mice (40)). Since TGF β 1 is also stored in large amount in platelets and Col1 particularly can induce platelet exocytosis (41), the presence of vascular Col1 in HCHWA-D (chapter 5) might enhance platelet recruitment and exocytosis, thereby providing local higher TGF β 1 and APP concentrations and further aggravating (or even initiating) the CAA pathology.

- Pathomechanisms such as enhanced formation of oA β , endothelial injuries or coagulation disorders could enhance endogenous TGF β response before stroke onset

Future perspectives

Pathomechanisms in HCHWA-D

A major question raised by this work is whether the Dutch mutation itself triggers an endogenous TGF β increase.

As described above, TGF β 1 cerebral overexpression in AD mice induces a CAA pathology increase and a parenchymal amyloid reduction. Intriguingly, a CAA phenotype is consistently induced in transgenic animals upon insertion of Dutch mutation in the APP sequence (APP Dutch (42), APP Dutch/APP23 (43); APP Dutch/BACE1 (44); APP-E693Q/PS1 Δ exon9 (27); Knock-In model APP DSL (45)). TGF β 1 levels in those models would be interesting to measure in order to know whether the insertion of the Dutch mutation leads to an endogenous TGF β 1 increase as proposed in this thesis.

The direct effect of the Dutch A β peptide on TGF β -related mechanisms could be further tested experimentally. As described in this chapter, some biological processes might only be triggered by specific A β species such as oA β s. Obtaining specific A β species *in vitro* using synthetic A β is problematic because A β peptide is highly unstable (personal observations). Instead, endogenous A β produced by iPS patient-derived cells, combined with recent progress in organoids or organ-on-chip technologies, opens new possibilities. Once established, an endogenous TGF β increase, for example, in patient-derived astrocyte/neuronal cocultures would be an interesting phenotype for further studies into pathomechanisms or therapeutic targets.

Biomarkers of cerebrovascular fibrosis

New biomarkers of CAA pathology in blood and cerebrospinal fluid (CSF) would be highly valuable to detect the disease onset, improve the clinical diagnosis and understand underlying pathomechanisms.

Results from this thesis suggest that biomarkers of cerebrovascular fibrosis such as direct measure of TGF β levels or detection of downstream pro-fibrotic components (such as FN1, Serpine1 and OPN) could be of interest. Brain TGF β levels in asymptomatic mutation carriers and in post-stroke patients have not been determined to date. In mutation carriers, detection of TGF β levels might indicate the onset of brain injuries (see **Graphic summary**) and help in defining windows for therapeutic intervention. For clinical practice, biomarkers of cerebrovascular fibrosis could help to estimate disease severity and progression. Finally, monitoring such biomarkers in therapeutic intervention studies will add insight into safety, effect and effectiveness of drugs in clinical trials.

Developing biomarkers of cerebrovascular fibrosis would help to better estimate the onset and progression of CAA pathology in HCHWA-D but also in other CAA-related vasculopathy and small vessel diseases where TGF β plays a central role as well (46,47).

- Developing biomarkers of cerebrovascular fibrosis will help understanding the disease in its initial phase, improving diagnostic tools and defining the best therapeutic strategies for HCHWA-D
- Such biomarkers are of interest for other CAA pathologies

Therapeutics intervention

TGF β lowering therapies

TGF β pathway is a double-edged sword

TGF- β is key in tissue homeostasis and disruption of the TGF β pathway has been implicated in numerous human diseases. Activation of the TGF β pathway in systemic diseases (such as atherosclerosis) has beneficial anti-inflammatory properties as well as detrimental profibrotic effects. Similar dual effects have been observed in the CNS. In animal models, TGF β 1 increase is neuroprotective, especially acutely after injury (48–51). However, chronically high levels cause extensive cerebrovascular fibrosis as described in this thesis.

Considerations with TGF β lowering therapies

Given the short-term beneficial effect of TGF β on neuroprotection, TGF β lowering therapies should be considered with care. *In vivo* pharmacological tuning of TGF β in APP mice is feasible (52–54). In symptomatic patients, monitoring intracerebral TGF β to prevent high chronic levels could be beneficial. Indeed patients suffering from recurrent strokes most probably have higher TGF β levels than pre-symptomatic mutation carriers. In the

context of stroke, acute TGF β upregulation restricts the infarct size in mouse models (49,55). However, in ageing animals, the TGF β response is stronger and last longer (56,57) resulting in more long-term detrimental effects. Maintaining TGF β levels within a certain range might therefore slow down CAA severity and disease progression. In practice, strategies targeting the downstream ECM modulators and pro-fibrotic genes would be a safer option than interfering with the TGF β signaling. For example PSTG2 (COX-2) inhibitor celecoxib can lower FN1 and Col1 expression and attenuate the vessel wall thickness in stroke-prone spontaneously hypertensive rats (58).

Amyloid lowering therapy

Anti-A β immunotherapy

Two strategies of A β vaccination have been tried in AD clinical trial, either active immunization with A β species to trigger a host immune response, or passive immunization by direct infusion of anti-A β antibodies.

But although anti-A β immunotherapies are effective in removing parenchymal A β , an increase in CAA pathology is observed, implicating a TGF β upregulation. Amyloid-related imaging abnormalities (or ARIA) in amyloid-modifying therapeutic trials are a major concern and multiple studies indicate that vascular amyloid is the common pathophysiological mechanism behind ARIA (59). At autopsy, A β vaccination consistently induced a removal of parenchymal plaques and an increase in amyloid vascular deposition in brains of clinical trial participants (60,61). Anti-A β immunotherapy in old AD transgenic mice had similar effects, with a worsening in CAA pathology and hemorrhages with longer treatment. Interestingly this phenotype is again triggered by a change in the inflammatory state characterized by TGF β upregulation (62).

A first immunotherapy trial for probable CAA patients with Ponezumab, an antibody-targeting A β 40, was launched by Pfizer in 2013 (NCT01821118; <https://clinicaltrials.gov>). After promising results in mouse models with CAA pathology (63), infusions of humanized ponezumab or placebo were evaluated for changes in occipital vascular reactivity by BOLD fMRI (primary endpoint). Although Ponezumab was well tolerated and showed drug-placebo differences, it did not meet the primary endpoint and the trial was stopped in 2016 in phase II (results not yet published; (64)). The pathological effect of this therapy is unknown with the current biomarkers and understanding of pathomechanisms. Based on the loss of cerebrovascular reactivity in the treated patients (65) one might speculate that the treatment resulted in a possible aggravation of the CAA pathology similarly to the AD clinical trial observations. In the light of this thesis and previous literature (66), the loss of cerebrovascular reactivity could be associated with an increase in TGF β mediated vascular fibrosis.

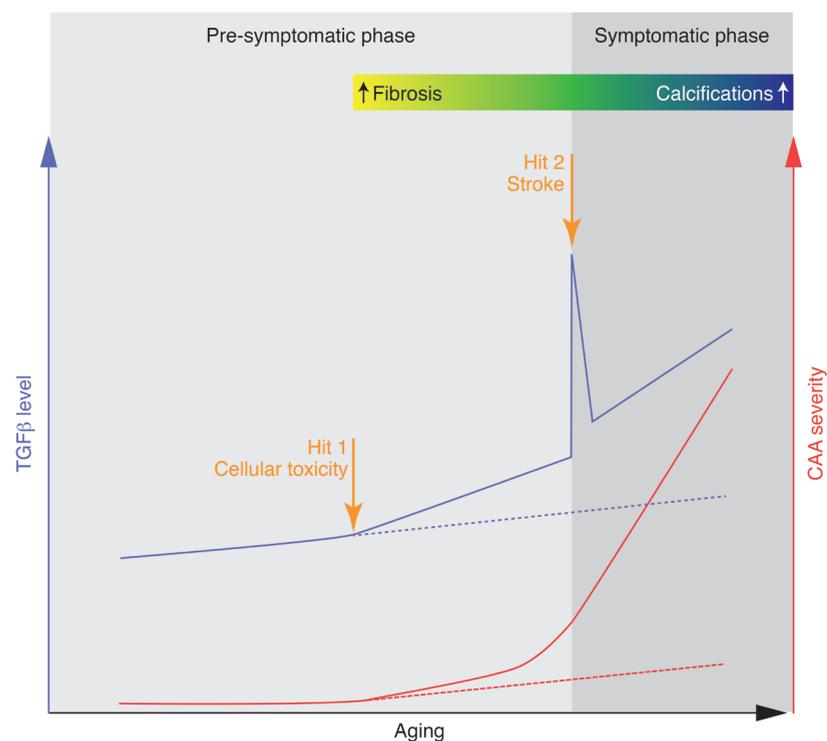
Other anti-A β strategies

Dutch amyloid seeds are harmful and irreversible due to a prion-like effect on WT A β (67), therefore an early intervention is preferable. One strategy, recently described for AD (68) and currently developed in our group for HCHWA-D, uses APP exon skipping to reduce the total A β secretion. Targeting cerebral genes with exon skipping is feasible and already in clinical trial for Huntington's Disease, where a recent clinical trial showed very promising results (69,70).

Alternative therapeutic strategies

Even though early anti-A β treatments are likely to be beneficial, therapeutic strategies uniquely focused on amyloid reduction will never completely prevent the CAA pathology in the context of vascular fibrosis. The complexity of CAA pathologies when amyloid deposition and cerebrovascular fibrosis are mixed is illustrated by studies in bitransgenic APP/TGF β (A/T) mice (71). Therapies which were beneficial in single APP or TGF β mice have a different outcome in A/T mice because vascular impairment caused by A β and TGF β are mechanistically different (72). In HCHWA-D and particularly in sporadic CAA, where ageing plays an important role, combinatorial therapeutic treatments simultaneously targeting soluble toxic amyloid species (such as Taxifolin targeting oA β s (73)) and preventing the cerebrovascular remodeling would in all likelihood be more effective than single target treatments.

- TGF β lowering therapies in brain should be considered with care
- Treatment to reduce the cerebrovascular fibrosis would be a safer option
- Early anti-A β intervention is preferable



Graphic summary: hypothetical pathomechanisms linking brain TGF β increase with CAA progression. An early response to cellular toxicity could initiate the TGF β increase (Hit 1) and trigger the vascular fibrosis in the pre-symptomatic phase. Acute stroke-related TGF β increase (Hit 2) could further accelerate the disease progression; leading in final stage to calcification of some angiopathic vessels.

References

- Vinters H V., Natté R, Maat-Schieman MLC, Van Duinen SG, Hegeman-Kleinn I, Welling-Graafland C, et al. Secondary microvascular degeneration in amyloid angiopathy of patients with hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA-D). *Acta Neuropathol.* 1998 Mar;95(3):235–44.
- Koemans EA, van Etten ES, van Opstal AM, Labadie G, Terwindt GM, Wermer MJH, et al. Innovative Magnetic Resonance Imaging Markers of Hereditary Cerebral Amyloid Angiopathy at 7 Tesla. *Stroke.* 2018 Apr; 49(6):1518–20.
- Mohler ER, Adam LP, McClelland P, Graham L, Hathaway DR. Detection of osteopontin in calcified human aortic valves. *Arterioscler Thromb Vasc Biol.* 1997 Mar;17(3):547–52.
- Mccullumsmith RE, Hammond JH, Shan D, Meador-Woodruff JH. Postmortem brain: An underutilized substrate for studying severe mental illness. *Neuropsychopharmacology.* 2014 Jan;39(1):65–87.
- Tomita H, Vawter MP, Walsh DM, Evans SJ, Choudary P V., Li J, et al. Effect of agonal and postmortem factors on gene expression profile: Quality control in microarray analyses of postmortem human brain. *Biol Psychiatry.* 2004 Feb 15;55(4):346–52.
- Vivien D, Ali C. Transforming growth factor- β signalling in brain disorders. *Cytokine Growth Factor Rev.* 2006;17(1–2):121–8.
- Buckwalter M, Pepper JP, Gaertner RF, Von Euw D, Lacombe P, Wyss-Coray T. Molecular and functional dissection of TGF- β 1-induced cerebrovascular abnormalities in transgenic mice. In: *Annals of the New York Academy of Sciences.* 2002. p. 87–95.
- Buckwalter MS, Wyss-Coray T. Modelling neuroinflammatory phenotypes in vivo. *J Neuroinflammation.* 2004 Jul 1;1(1):10.
- Wyss-Coray T, Lin C, Sanan DA, Mucke L, Masliah E. Chronic overproduction of transforming growth factor- β 1 by astrocytes promotes Alzheimer's disease-like microvascular degeneration in transgenic mice. *Am J Pathol.* 2000 Jan;156(1):139–50.
- Ueberham U, Ueberham E, Brückner MK, Seeger G, Gärtner U, Gruschka H, et al. Inducible neuronal expression of transgenic TGF- β 1 in vivo: Dissection of short-term and long-term effects. *Eur J Neurosci.* 2005 Jul;22(1):50–64.
- Maat-Schieman MLC, Yamaguchi H, Hegeman-Kleinn IM, Welling-Graafland C, Natté R, Roos RAC, et al. Glial reactions and the clearance of amyloid β protein in the brains of patients with hereditary cerebral hemorrhage with amyloidosis-Dutch type. *Acta Neuropathol.* 2004 May;107(5):389–98.
- van Duinen SG, Maat-Schieman MLC, Buijn JA, Haan J, Roos RAC, Wattendorff AR, et al. Cortical Tissue of Patients With Hereditary Cerebral-Hemorrhage With Amyloidosis (Dutch) Contains Various Extracellular-Matrix Deposits. *Lab Invest.* 1995 Aug;73(2):183–9.
- Wilhelmus MMM, Bol JGJM, van Duinen SG, Drukarch B. Extracellular matrix modulator lysyl oxidase colocalizes with amyloid-beta pathology in Alzheimer's disease and hereditary cerebral hemorrhage with amyloidosis-Dutch type. *Exp Gerontol.* 2013 Feb;48(2):109–14.
- Colton CA. Heterogeneity of microglial activation in the innate immune response in the brain. *J Neuroimmune Pharmacol.* 2009 Dec;4(4):399–418.

15. Heneka MT, Carson MJ, Khoury J El, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol.* 2015;14(4):388–405.
16. Natté R, Maat-Schieman MLC, Haan J, Bornebroek M, Roos RAC, Van Duinen SG. Dementia in hereditary cerebral hemorrhage with amyloidosis-Dutch type is associated with cerebral amyloid angiopathy but is independent of plaques and neurofibrillary tangles. *Ann Neurol.* 2001 Dec;50(6):765–72.
17. Hawkes CA, McLaurin J. Selective targeting of perivascular macrophages for clearance of -amyloid in cerebral amyloid angiopathy. *Proc Natl Acad Sci.* 2009;106(4):1261–6.
18. Town T, Laouar Y, Pittenger C, Mori T, Szekely CA, Tan J, et al. Blocking TGF- β -Smad2/3 innate immune signaling mitigates Alzheimer-like pathology. *Nat Med.* 2008;14(6):681–7.
19. Lifshitz V, Weiss R, Benromano T, Kfir E, Blumenfeld-Katzir T, Tempel-Brami C, et al. Immunotherapy of cerebrovascular amyloidosis in a transgenic mouse model. *Neurobiol Aging.* 2012 Feb;33(2):432.e1-432.e13.
20. Vinters H V. Inflammation complicates an “age-related” cerebral microangiopathy. *Can J Neurol Sci.* 2011 Jul;38(4):543–4.
21. Viola KL, Klein WL. Amyloid β oligomers in Alzheimer's disease pathogenesis, treatment, and diagnosis. *Acta Neuropathol.* 2015;129(2):183–206.
22. DiChiara T, DiNunno N, Clark J, Bu R Lo, Cline EN, Rollins MG, et al. Alzheimer's Toxic Amyloid Beta Oligomers: Unwelcome Visitors to the Na/K ATPase alpha3 Docking Station. *Yale J Biol Med.* 2017 Mar;90(1):45–61.
23. Kassler K, Horn AHC, Sticht H. Effect of pathogenic mutations on the structure and dynamics of Alzheimer's A beta 42-amyloid oligomers. *J Mol Model.* 2010 May;16(5):1011–20.
24. Miravalle L, Tokuda T, Chiarle R, Giaccone G, Bugiani O, Tagliavini F, et al. Substitutions at codon 22 of Alzheimer's abeta peptide induce diverse conformational changes and apoptotic effects in human cerebral endothelial cells. *J Biol Chem.* 2000 Sep 1;275(35):27110–6.
25. Ohshima Y, Taguchi K, Mizuta I, Tanaka M, Tomiyama T, Kametani F, et al. Mutations in the β -amyloid precursor protein in familial Alzheimer's disease increase A β oligomer production in cellular models. *Heliyon.* 2018;(October 2017):e00511.
26. Sian a K, Frears ER, El-Agnaf OM, Patel BP, Manca MF, Siligardi G, et al. Oligomerization of beta-amyloid of the Alzheimer's and the Dutch-cerebral-haemorrhage types. *Biochem J.* 2000;349:299–308.
27. Gandy S, Simon AJ, Steele JW, Lublin AL, Lah JJ, Walker LC, et al. Days to criterion as an indicator of toxicity associated with human Alzheimer amyloid-beta oligomers. *Ann Neurol.* 2010 Aug;68(2):220–30.
28. Umeda T, Tomiyama T, Sakama N, Tanaka S, Lambert MP, Klein WL, et al. Intraneuronal amyloid beta oligomers cause cell death via endoplasmic reticulum stress, endosomal/lysosomal leakage, and mitochondrial dysfunction in vivo. *J Neurosci Res.* 2011 Jul;89(7):1031–42.
29. Viana RJS, Nunes a F, Castro RE, Ramalho RM, Meyerson J, Fossati S, et al. Tauroursodeoxycholic acid prevents E22Q Alzheimer's A β toxicity in human cerebral endothelial cells. *Cell Mol Life Sci.* 2009;66(6):1094–104.
30. Tan X-L, Xue Y-Q, Ma T, Wang X, Li JJ, Lan L, et al. Partial eNOS deficiency causes spontaneous thrombotic cerebral infarction, amyloid angiopathy and cognitive impairment. *Mol Neurodegener.* 2015;10(1):24.
31. Paris D, Ait-Ghezala G, Mathura VS, Patel N, Quadros A, Laporte V, et al. Anti-angiogenic activity of the mutant Dutch A(beta) peptide on human brain microvascular endothelial cells. *Brain Res Mol Brain Res.* 2005 May 20;136(1–2):212–30.
32. Solito R, Corti F, Fossati S, Mezhericher E, Donnini S, Ghiso J, et al. Dutch and Arctic mutant peptides of beta amyloid(1-40) differentially affect the FGF-2 pathway in brain endothelium. *Exp Cell Res.* 2009 Mar 1;315(3):385–95.
33. Liddel SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature.* 2017;541(7638):481–7.
34. Diniz LP, Tortelli V, Matias I, Morgado J, Bergamo Araujo AP, Melo HM, et al. Astrocyte Transforming Growth Factor Beta 1 Protects Synapses against Abeta Oligomers in Alzheimer's Disease Model. *J Neurosci.* 2017 Jul;37(28):6797–809.
35. van der Wal EA, Gomez-Pinilla F, Cotman CW. Transforming growth factor-beta 1 is in plaques in Alzheimer and Down pathologies. *Neuroreport.* 1993 Jan;4(1):69–72.
36. Mousseau DD, Chapelsky S, De Crescenzo G, Kirkitadze MD, Magoon J, Inoue S, et al. A direct interaction between transforming growth factor (TGF)-betas and amyloid-beta protein affects fibrillogenesis in a TGF-beta receptor-independent manner. *J Biol Chem.* 2003 Oct 3;278(40):38715–22.
37. Huang SS, Huang FW, Xu J, Chen S, Hsu CY. Amyloid beta-Peptide Possesses a Transforming Growth Factor-beta Activity *. 1998;273(42):27640–4.
38. Bornebroek M, Von Dem Borne PAK, Haan J, Meijers JCM, Van Nostrand WE, Roos RAC. Binding of amyloid β precursor protein to coagulation factor XIa in vivo may favour haemorrhagic stroke. *J Neurol.* 1998 Feb;245(2):111–5.
39. Rozemuller AJ, Roos RA, Bots GT, Kamphorst W, Eikelenboom P, Van Nostrand WE. Distribution of beta/A4 protein and amyloid precursor protein in hereditary cerebral hemorrhage with amyloidosis-Dutch type and Alzheimer's disease. *Am J Pathol.* 1993;142(5):1449–57.
40. Gowert NS, Donner L, Chatterjee M, Eisele YS, Towhid ST, Münzer P, et al. Blood platelets in the progression of Alzheimer's disease. *PLoS One.* 2014;9(2).
41. Alberio L, Dale GL. Flow cytometric analysis of platelet activation by different collagen types present in the vessel wall. *Br J Haematol.* 1998 Sep;102(5):1212–8.
42. Herzig MC, Winkler DT, Burgermeister P, Pfeifer M, Kohler E, Schmidt SD, et al. A β is targeted to the vasculature in a mouse model of hereditary cerebral hemorrhage with amyloidosis. *Nat Neurosci.* 2004 Oct;7(9):954–60.
43. Herzig MC, Eisele YS, Staufienbiel M, Jucker M. E22Q-mutant A β peptide (A β Dutch) increases vascular but reduces parenchymal A β deposition. *Am J Pathol.* 2009 Mar;174(3):722–6.
44. Herzig MC, Paganetti P, Staufienbiel M, Jucker M. BACE1 and mutated presenilin-1 differently modulate A β 40 and A β 42 levels and cerebral amyloidosis in APPDutch transgenic mice. *Neurodegener Dis.* 2007 Jan;4(2–3):127–35.
45. Li H, Guo Q, Inoue T, Polito V a, Tabuchi K, Hammer RE, et al. Vascular and parenchymal amyloid pathology in an Alzheimer disease knock-in mouse model: interplay with cerebral blood flow. *Mol Neurodegener.* 2014;9:28.
46. Pezzini A, Del Zotto E, Volonghi I, Giossi A, Costa P, Padovani A. Cerebral Amyloid Angiopathy: A Common Cause of Cerebral Hemorrhage. *Curr Med Chem.* 2009 Jul;16(20):2498–513.

47. Müller K, Courtois G, Ursini MV, Schwaninger M. New Insight Into the Pathogenesis of Cerebral Small-Vessel Diseases. *Stroke*. 2017;STROKEAHA.116.012888.
48. Flanders KC, Ren RF, Lippa CF. Transforming growth factor-betas in neurodegenerative disease. *Prog Neurobiol*. 1998;54(1):71–85.
49. Doyle KP, Cekanaviciute E, Mamer LE, Buckwalter MS. TGF β signaling in the brain increases with aging and signals to astrocytes and innate immune cells in the weeks after stroke. *J Neuroinflammation*. 2010 Jan;7(1):62.
50. Cekanaviciute E, Fathali N, Doyle KP, Williams AM, Han J, Buckwalter MS. Astrocytic transforming growth factor-beta signaling reduces subacute neuroinflammation after stroke in mice. *Glia*. 2014;62(8):1227–40.
51. Dobolyi A, Vincze C, Pál G, Lovas G. The neuroprotective functions of transforming growth factor Beta proteins. *Int J Mol Sci*. 2012 Jan;13(7):8219–58.
52. Zhang ZY, Li C, Zug C, Schluesener HJ. Icaritin ameliorates neuropathological changes, TGF- β 1 accumulation and behavioral deficits in a mouse model of cerebral amyloidosis. *PLoS One*. 2014;9(8).
53. Sachdeva AK, Chopra K. Lycopene abrogates A β (1-42)-mediated neuroinflammatory cascade in an experimental model of Alzheimer's disease. *J Nutr Biochem*. 2015;26(7):736–44.
54. Li C, Zug C, Qu H, Schluesener H, Zhang Z. Hesperidin ameliorates behavioral impairments and neuropathology of transgenic APP/PS1 mice. *Behav Brain Res*. 2015;281:32–42.
55. Song L, Liu F, Liu C, Li X, Zheng S, Li Q, et al. Neuroprotective effects of SMADs in a rat model of cerebral ischemia/reperfusion. *Neural Regen Res*. 2015;10(3):438.
56. Lively S, Schlichter LC. Age-Related Comparisons of Evolution of the Inflammatory Response After Intracerebral Hemorrhage in Rats. *Transl Stroke Res*. 2012;3(SUPPL. 1):132–46.
57. Buga AM, Margaritescu C, Scholz CJ, Radu E, Zelenak C, Popa-Wagner A. Transcriptomics of post-stroke angiogenesis in the aged brain. *Front Aging Neurosci*. 2014;6(MAR):1–20.
58. Tang J, Xiao W, Li Q, Deng Q, Chu X, Chen Y, et al. A Cyclooxygenase-2 Inhibitor Reduces Vascular Wall Thickness and Ameliorates Cognitive Impairment in a Cerebral Small Vessel Diseases Rat Model. Vol. 12, *Current Alzheimer Research*. 2015. p. 704–10.
59. Sperling RA, Jack CRJ, Black SE, Frosch MP, Greenberg SM, Hyman BT, et al. Amyloid-related imaging abnormalities in amyloid-modifying therapeutic trials: recommendations from the Alzheimer's Association Research Roundtable Workgroup. *Alzheimers Dement*. 2011 Jul;7(4):367–85.
60. Zotova E, Bharambe V, Cheaveau M, Morgan W, Holmes C, Harris S, et al. Inflammatory components in human Alzheimer's disease and after active amyloid-42 immunization. *Brain*. 2013 Aug 13;
61. Boche D, Zotova E, Weller RO, Love S, Neal JW, Pickering RM, et al. Consequence of A β immunization on the vasculature of human Alzheimer's disease brain. *Brain*. 2008 Dec;131(Pt 12):3299–310.
62. Wilcock DM, Rojiani A, Rosenthal A, Subbarao S, Freeman MJ, Gordon MN, et al. Passive immunotherapy against A β in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhemorrhage. Vol. 1, *Journal of Neuroinflammation*. London; 2004. p. 24.
63. Bales KR, O'Neill SM, Pozdnyakov N, Pan F, Caouette D, Pi Y, et al. Passive immunotherapy targeting amyloid- β reduces cerebral amyloid angiopathy and improves vascular reactivity. *Brain*. 2016;139(2):563–77.
64. Banerjee G, Carare R, Cordonnier C, Greenberg SM, Schneider JA, Smith EE, et al. The increasing impact of cerebral amyloid angiopathy: Essential new insights for clinical practice. *J Neurol Neurosurg Psychiatry*. 2017;88(11):982–94.
65. Leurent C, Goodman JA, Zhang Y, He P, Polimeni JR, Gurol ME, et al. Immunotherapy with ponezumab for probable cerebral amyloid angiopathy. *Ann Clin Transl Neurol*. 2019;6(4):795–806.
66. Tong X-K. Vascular Remodeling versus Amyloid β -Induced Oxidative Stress in the Cerebrovascular Dysfunctions Associated with Alzheimer's Disease. *J Neurosci*. 2005 Nov 30;25(48):11165–74.
67. Condello C, Lemmin T, Stöhr J, Nick M, Wu Y, Maxwell AM, et al. Structural heterogeneity and intersubject variability of A β in familial and sporadic Alzheimer's disease. *Proc Natl Acad Sci*. 2018;201714966.
68. Chang JL, Hinrich AJ, Roman B, Norrbom M, Rigo F, Marr RA, et al. Targeting Amyloid-beta Precursor Protein, APP, Splicing with Antisense Oligonucleotides Reduces Toxic Amyloid-beta Production. *Mol Ther*. 2018 Jun;26(6):1539–51.
69. Rutten JW, Dauwerse HG, Peters DJM, Goldfarb A, Venselaar H, Haffner C, et al. Therapeutic NOTCH3 cysteine correction in CADASIL using exon skipping: In vitro proof of concept. *Brain*. 2016 Apr;139(4):1123–35.
70. Toonen LJA, Rigo F, van Attikum H, van Roon-Mom WMC. Antisense Oligonucleotide-Mediated Removal of the Polyglutamine Repeat in Spinocerebellar Ataxia Type 3 Mice. *Mol Ther Nucleic Acids*. 2017 Sep;8:232–42.
71. Ongali B, Nicolakakis N, Lecrux C, Aboukassim T, Rosa-Neto P, Papadopoulos P, et al. Transgenic mice overexpressing APP and transforming growth factor- β 1 feature cognitive and vascular hallmarks of Alzheimer's disease. *Am J Pathol*. 2010 Dec;177(6):3071–80.
72. Hamel E. Cerebral Circulation: Function and Dysfunction in Alzheimer's Disease. *J Cardiovasc Pharmacol*. 2015;65(4):317–24.
73. Saito S, Yamamoto Y, Maki T, Hattori Y, Ito H, Mizuno K, et al. Taxifolin inhibits amyloid- β oligomer formation and fully restores vascular integrity and memory in cerebral amyloid angiopathy. *Acta Neuropathol Commun*. 2017;5(1):26.